Organization versus Activation: The Role of Endocrine-disrupting Contaminants (EDCs) during Embryonic Development in Wildlife

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Many environmental contaminants disrupt the vertebrate endocrine system. Although they may be no more sensitive to endocrine-disrupting contaminants (EDCs) than other vertebrates, reptiles are good sentinels of exposure to EDCs due to the lability in their sex determination. This is exemplified by a study of alligators at Lake Apopka, Florida, showing that EDCs have altered the balance of reproductive hormones resulting in reproductive dysfunction. Such alterations may be activationally or organizationally induced. Much research emphasizes the former, but a complete understanding of the influence of EDCs in nature can be generated only after consideration of both activational and organizational alterations. The organizational model suggests that a small quantity of an EDC, administered during a specific period of embryonic development, can permanently modify the organization of the reproductive, immune, and nervous systems. Additionally, this model helps explain evolutionary adaptations to naturally occurring estrogenic compounds, such as phytoestrogens. — Environ Health Perspect 103(Suppl 7):157–164 (1995)

Key words: endocrine disrupting contaminants, reptiles, steroids, estrogen, activation, organization, embryo

Introduction

Environmental contaminants have posed a major threat to wildlife health since the onset of the industrial age. The focus of our concern on the health consequences of environmental pollution have, in the last three decades, been on lethal, carcinogenic, and extreme teratogenic manifestations. Evidence from a number of sources suggests that another mechanism, endocrine-disruption, must also be examined (*1*).

There is good evidence that man-made factors in the environment act as hormones or antihormones. It was this concern that led the National Institute of Environmental Health Sciences to organize a symposium—"Estrogens in the Environment"—in 1979 to address growing concern that many estrogenic compounds, such as diethylstilbestrol (DES) used in the animal science industry and the pesticide DDT, were being released into the environment (*2*). Over a decade later, the World Wildlife Fund (*3*) organized a conference that addressed the premise that xenobiotic compounds did not act solely as estrogens but were also agonists and antagonists of other hormones that disrupted the endocrine system of developing embryos. Disruption of embryonic development produced permanent modifications in the reproductive, immunological, and neurological capabilities of future populations and did so by mechanisms other than gene mutation. The World Wildlife Fund meeting generated a consensus statement by the attending scientists which read, "We are certain of the following: a large number of man-made chemicals that have been released into the environment...have the potential to disrupt the endocrine systems of animals, including humans" (*3*). To further assess the damage done to wildlife populations, it is essential that we expand the wildlife models currently in use. One group of vertebrates, the reptiles, can provide important new insight due to a number of attributes associated with their system of sex determination.

Reptiles as Models for the Study of Endocrine-disrupting Xenobiotics

Many crocodilian (including alligators) and turtle species exhibit environmentally (temperature) determined sex, and this environmental influence can be overcome (sex reversal) by treating eggs with estrogen. Genetic factors associated with sex determination in reptiles, and thus gonadal development, are still under study, but the role of temperature and sex steroids has been extensively examined (*4–7*). In alligators, the temperature of incubation at specific critical periods of embryonic development triggers the determination of sex (*4,7*). Incubation temperature induces an all-or-none response so that embryos are either males or females, with few intersexes produced (*8*).

Studies have shown that alligators and several turtle species can exhibit sex reversal (male to female) if developing embryos are exposed to an estrogenic compound during a specific period of development, usually the second third of the embryonic period. This critical period represents a window of heightened vulnerability to estrogenic compounds. Thus, compounds with a short half-life in the environment need only be present during this window to have permanent developmental effects. For instance, a single pulse of estradiol treatment given to alligator (*9*) or turtle (*10,11*) eggs incubated at male-producing temperatures can induce the production of apparently normal

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Abbreviations used: DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; EDCs, endocrine-disrupting contaminants; PCBs, polychlorinated biphenyls; DDE, dichlorodiphenyldichloroethylene; E_2, 17β-estradiol; IGF-I, insulinlike growth factor I; SBG, sex steroid-binding globulin; T, testosterone.

females. Intersex individuals are produced when the concentration of an estrogenic compound is below a specific threshold. That is, with intermediate treatment levels, an embryo may exhibit Müllerian (female) ducts and a testis or even an ovotestis. Turtle eggs exposed (painted or injected) to estradiol or other estrogenic compounds (polychlorinated biphenyls [PCBs]) show such partial reversal. Bergeron et al. (*12*) observed that 2′,4′,5′-trichloro-4-biphenylol induced 100% male to female sex reversal (based on histological examination of gonads and internal ducts) in the red-eared turtle (*Trachemys scripta elegans*), whereas treatment with 2′,3′,4′,5′-tetrachloro-4-biphenylol stimulated total sex reversal in 50% of the embryos and partial sex reversal (intersex) in 21% of the embryos. Interestingly, red-belly turtle neonates (*Pseudemys nelsoni*) from Lake Apopka, Florida, contaminated with a number of endocrine-disrupting contaminants (EDCs), the most common being *p,p′*-DDE, have primarily normal ovaries or abnormal ovotestes—very few animals exhibit morphologically normal testes (TS Gross and LJ Guillette, unpublished data). Moreover, chorioallantoic fluid concentrations of 17β-estradiol (E_2) and testosterone (T) indicate that no turtle hatchling from Lake Apopka produces a normal androgen synthesis pattern (TS Gross and LJ Guillette, unpublished data). Similar observations have been reported for the alligators living in Lake Apopka (*13,14*). Both males and female juvenile alligators exhibited abnormal plasma sex steroid concentrations, with males from Lake Apopka having greatly reduced plasma T concentrations similar to that of females from either the contaminated (Lake Apopka) or control (Lake Woodruff) lakes. The juvenile female alligators from Lake Apopka had elevated plasma E_2 concentrations compared to the females from the control lake. Testes from Lake Apopka juvenile males make elevated levels of E_2 but normal levels of T *in vitro*, suggesting that the aromatase enzyme system, essential for estrogen synthesis, is upregulated in males (*14*). Treatment of alligator eggs with either DDE or estradiol induces similar results, with complete sex reversal or intersexes and abnormal plasma steroid concentrations observed (TS Gross and LJ Guillette, unpublished data).

These studies suggest that reptiles represent excellent model species to determine the extent of contamination of an ecosystem due to the lability of their sex determination in response to the presence of steroids, steroid-mimicking compounds, or steroid synthesis-disrupting compounds (*12,15,16*). In addition to their labile sex determination, reptiles such as turtles and alligators represent species that feed at various levels in the food web and live for extensive periods. Many species also show strong site affinity, allowing the examination of specific wetland regions. These attributes, and those of other species, will help provide general answers on how EDCs affect wildlife populations. However, these studies will not address the full extent of the EDCs threat to wildlife health unless all life stages are examined, especially the period of embryonic development.

Organization versus Activation

If embryos are a major life stage affected by EDCs, it is important to address why this stage is so susceptible to factors characterized as weak estrogens and antiestrogens when compared to the native estrogen 17β-estradiol. In this discussion, we address a number of biological principles we feel should provide a framework for future EDC research. Obviously, embryos are small and exhibit high rates of mitosis, which makes them sensitive to environmental perturbations, but additional factors contribute to an embryo's organizational response to EDC exposure: *a*) critical periods of sensitivity during embryonic organization, *b*) bioaccumulation versus degradation and secretion, and *c*) free versus bound hormone. These principles are important as they suggest that all EDCs have the potential to significantly modify the organization of a developing embryo. We will address these issues in relation to the organizational versus activational framework and provide examples from wildlife.

It was only recently that studies in the field of developmental biology began to appreciate the diverse roles of hormones during early embryonic development (*17*). The pervasive view has held that hormones could not influence embryonic development until a source of hormones was present. Early studies, for example, clearly showed that androgens and Müllerian inhibiting hormone were essential for development of mammalian, male reproductive duct systems, but this influence occurred only after a source of these hormones, the testes, formed (*18*). However, recent studies have begun to show that many embryonic cells exhibit receptors for various hormones significantly before these hormones are synthesized by embryonic sources. For example, developing chick gastrulas exhibit receptors for insulinlike growth factor I (IGF-I) on the day of laying (*19*), but an embryonic source of this hormone is not present until several days later (*20*). Similar observations have now been made in frogs (*21*). These data suggest that early embryos would be capable of responding to a hormonal signal if a signal were present.

Is there a source of hormone that might interact with the receptors present before an embryonic source of hormone? IGF-I of maternal origin has been identified in the yolk of chicken eggs (*22*) and in the yolk and albumen of alligator eggs (*23*). Likewise, the yolk of teleost fish is a rich source of maternal thyroid (*24*) and various steroid (*25*) hormones, and alligator yolk has abundant steroid hormone concentrations at the time of ovulation (Guillette et al., unpublished data). Eggs of most vertebrate species have been shown to possess significant quantities of various environmental contaminants, many of which act as hormone mimics and bind with specific hormone receptors (*26,27*). This is especially true for turtles and alligators that lay large, yolky eggs (*28–32*). The ability to bioaccumulate and mimic hormones makes endocrine-disrupting contaminants potent modifiers of embryonic development.

The Importance of Contaminant Exposure during Embryonic Organization. The concepts of organization versus activation have been useful in explaining the role of hormones in the differentiation of vertebrate sexual dimorphism, whether morphological, physiological, or behavioral (*33*). As originally defined, organizational effects occur early in an individual's lifetime and induce permanent effects, whereas activational effects usually are transitory actions occurring during adulthood (*34*). For example, androgens organize mammalian embryos by stimulating the development of the male reproductive duct/glandular system as well as the external genitalia (Figure 1). Likewise, androgens stimulate growth and secretory activity of glands associated with the male reproductive tract, an activational effect (Figure 1). In their original presentation of the organizational/activational concept, Phoenix et al. (*34*) examined the role of prenatal testosterone treatment on subsequent adult behavior of guinea pigs and suggested that three main criteria defined this concept. Organizational effects *a*) were permanent, *b*) occur early in life, usually just before or after birth, and *c*) occur

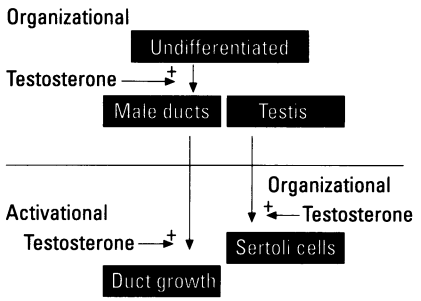

Figure 1. A simple representation of the organizational versus activational model. The presence of testosterone early in development induces the differentiation of the Wolffian duct so as to form the male internal reproductive duct system (*18*). The source of testosterone for this transformation is the testis. In mammals, testicular formation is apparently due to a genetically expressed signal that does not involve a steroid hormone. In contrast, in reptiles, steroids or enzyme inhibitors (antiaromatase) can modify embryonic development and gonadal formation, thus having an organizing effect at this level (*16,35*). Thus, in mammals and reptiles, testosterone-induced reproductive duct development is said to be an organizing effect whereas the stimulation of reproductive duct growth and secretion by testosterone after puberty is activational. When initially presented, organizational effects were believed to occur before birth, but it is clear that these effects can continue throughout life, especially prior to puberty [e.g., stimulation of Sertoli cell number during neonatal development (*36*)].

during a critical or sensitive period. The three original criteria have been extended by subsequent researchers. The first extension states that permanent organizational effects imply structural changes in tissues or organs; the second extension concludes that organizational effects produce sexual dimorphism (*33*). However, it is simplistic to assume that an individual is completely organized at birth. Current research indicates that steroid hormones have organizing effects on the neonate [e.g., stimulation of Sertoli cell number (*36*)] and even into adulthood [e.g., changes in mammary tissue with pregnancy (*37*)].

Much of the work, both theoretical and experimental, examining the organizational versus activational roles of hormones has been focused in the field of neurobiology and behavior (*33*). However, these same concepts are also central to the role of environmental EDCs. Currently, many studies examine the activational effects of hormone-mimicking environmental contaminants, primarily in adult model systems. In contrast, we must emphasize the organizational effects of these endocrine mimics if we are to assess their complete impact on environmental health. It is, in fact, the reorganization of the embryo by exposure to EDCs that is a major concern. There are well-known examples of this phenomenon.

The best known and most intensively studied example is the DES daughter/son syndrome. The DES syndrome was partially recognized and understood because of a preexisting model using prenatally or perinatally estrogen-exposed laboratory rodents (*38*). In rodents, exposure to estrogenic compounds during *in utero* development or immediately after birth results in pathological changes of the reproductive tract, as well as functional differences at puberty and throughout adulthood. Similar estrogenic effects have also been demonstrated on the immune (*39*) and neuroendocrine (*40*) systems.

Many of the observed DES-induced modifications to the reproductive system are morphologically subtle but result in major functional changes. A number of studies have documented that the reproductive tract of both male and female mice exposed neonatally to DES exhibit protein and gene expression patterns that are unique when compared to controls (*41–43*). Exposure of neonatal male mice to DES produced a significant organizational effect so that, at sexual maturity, the seminal vesicle stimulated secondarily with estrogen could synthesize lactoferrin, a protein not normally produced by this gland (*44*). Secretion of lactoferrin was at a level similar to that seen in normal uterine tissue. Perinatal exposure of mice to DES also induces organizational modifications of a very subtle type; that is, it modifies the types and abundance of receptors on various tissues of the reproductive system (*38*). Although changes in receptor type and abundance can be considered subtle, developmental abnormalities of this type may be the basis for infertility or reproductive cancers (*38*).

Similar to the reproductive effects caused by DES, immune modification resulting from neonatal DES exposure occurs during a critical period. Whereas adult exposure to estrogens temporarily inhibits many aspects of the immune system (*45*), neonatal exposure to DES causes a persistent impairment of several immune parameters, including reduced delayed-hypersensitivity response, decreased *in vitro* mitogen response, and depressed graft versus host reaction (*46,47*). The developmental stage during which exposure occurs seems to be critical for determining persistence of estrogenic effects. For example, DES treatment of mice during the first 5 days after birth causes immune depression evident at 17 months of age, whereas treatments on days 6 through 10 had no discernible effects later in life (*47*). The mechanisms of the action of estrogens on the immune system appear to involve both lymphoid and nonlymphoid tissues. Estrogen receptors are present at low levels in lymphoid cells and near uterine levels in thymic epithelium (*39*). EDCs may alter estrogen receptor levels in immune tissues, similar to receptor changes of the reproductive system. However, to our knowledge, modifications in steroid receptor number in immune tissues following embryonic exposure to EDCs have not been investigated.

A complication when examining the immune or reproductive systems is the interwoven nature of their function. The bidirectional communication between the neuroendocrine and immune systems has been the subject of many recent reviews (*48*). One such manifestation of this relationship may be the relationship between immunotoxicity and estrogenicity. Immunotoxicity of estrogens correlates with estrogenicity as measured by uterotrophic activity (*39*). The interactions between the neuroendocrine and immune systems appear to be temporally restricted in a manner similar to the increased sensitivity to EDCs during windows in the organizational phase of development. Neonatally thymectomized mice, as well as congenitally athymic mice, display a profoundly altered endocrine system (*49*). Endocrine irregularities associated with this T-lymphocyte deficiency include delays in puberty in females, alterations in normal adenohypophysis formation, alterations in adrenal gland formation, and abnormal circulating levels of gonadal hormones. These conditions are often developmentally fixed and fail to normalize despite successful restoration of immunocompetence by lymphoid replacement later in life.

Interestingly, it is also important to note that many organizational modifications do not become apparent until later in life. For example, exposure to elevated concentrations of estrogens during puberty and adult life appears to contribute to an increase in the severity of DES-induced embryonic abnormalities. For example, a major symptom of the DES daughter syndrome is the occurrence of vaginal clear-cell carcinoma in young women (*50*). This phenomenon only occurs after puberty when plasma estrogen concentrations are elevated and stimulate reproductive tract growth. In mice, postnatal estrogen exposure significantly increases the severity of

the reproductive abnormalities induced by prenatal estrogen exposure (*42,51,52*).

Examples of organizational effects in wildlife species have been noted as well (Table 1). The pioneering studies of Fry and Toone (*57*) demonstrated that exposure of seabird eggs to DDT concentrations, comparable to those found in contaminated eggs obtained in wild nests, induced the development of ovarian tissue and oviducts in male embryos (*57*). Similar EDC-induced gonadal abnormalities have been noted in some fish populations (*62*). A recent study demonstrated that PCBs will induce sex reversal—male to female—in freshwater turtles with the development of apparently normal ovaries and oviducts (*12*). Organizational modifications have also been reported for alligators collected from a lake, Lake Apopka, known to have wildlife and eggs with elevated *p,p'*-DDE concentrations. Gonads of male and female juvenile alligators are permanently modified morphologically and physiologically after exposure to this EDC (*13,14*). Although the specific time of embryonic exposure is unknown, as these are wild eggs possessing elevated contaminant levels at ovulation, the nature of the reproductive abnormalities (polyovular follicles, poorly organized testes, small phalli, and altered steroid secretion) indicates that exposure probably occurred throughout the *in ovo* period, inducing multiple organizational defects as observed. Other wildlife species feeding in contaminated food webs exhibit developmental abnormalities characteristic of endocrine disruption, and many adults show reproductive, immune, and neurological abnormalities that may represent organizational or activational effects (Table 1). A specific cause–effect relationship is often difficult to recognize in wildlife species, but laboratory studies confirm that *in ovo* or *in utero* exposure to EDCs can cause irreversible alterations to the reproductive systems of wildlife species.

It was not until the medical and scientific community began to appreciate the organizational influences of DES on the developing mammalian embryo that the true magnitude of the DES daughter/son syndrome became clear (*38*). If we are to assess the threat of endocrine-disrupting contaminants to wildlife and human populations, then we must examine both activational and organizational effects of EDCs on developing embryos.

Bioaccumulation and Evolutionary Adaptation to Endocrine-disrupting Contaminants. One of the legacies of environmental pollution is the bioaccumulation and biomagnification of contaminants within the animals feeding at various levels of the food chain. Lipid-soluble pollutants are stored in fat reserves and, upon mobilization during reproductive events, developing embryos are exposed to the bioaccumulated contaminants. Females with large, yolky eggs use the energy stores in fat reserves to synthesize and store various compounds in the oocyte, and these compounds are later used for embryonic development and growth. Hormones such as growth factors (e.g., insulinlike growth factor-I), steroids (17β-estradiol, testosterone), thyroxin, and vitamins are deposited in the yolk for use during embryonic development, but EDCs such as PCBs, DDT, DDE, and dioxin, to name a few, also are deposited in the eggs because all females have bioaccumulated EDCs. Similarly, embryos developing *in utero* are exposed to hormones and EDCs via uterine secretion and placental transfer.

It is clear that developing embryos can be exposed to EDCs, but it has been argued that this exposure is innocuous because embryos are normally exposed to exogenous (plant) estrogenic sources. This argument is supported by the observation that herbivores grazing on various plants containing phytoestrogens can transport these plant secondary compounds to their developing young. A number of these phytoestrogens are known to interact with the endocrine system and disrupt reproduction.

Table 1. Organizational and presumed activational effects of environmental endocrine-disrupting contaminants (EDCs) in wildlife species.[a]

Species	Organizational effect[b]	Activational effect[b]	EDCs[c]	References
Fish				
Salmon	Premature sexual maturity Loss of sexual dimorphism ↑ Embryo mortality	↓ Fertility ↓ 17β-Estradiol ↓ Dihydrooxyprogesterone	PCBs DDT Dioxins Furans Metals	(*53*)
Mosquito-fish	?	Masculinization of females Anal fin modifications Mating behavior	Kraft mill effluent	(*54*)
Trout	?	↑ Plasma vitellogenin (yolk protein) in males	Sewage works effluent	(*55*)
White croaker	↑ Embryonic mortality	↓ Fecundity and fertility ↑ Ovarian follicular atresia	DDT	(*56*)
Reptiles				
Snapping turtle	↑ Embryonic deformities	?	PCBs Dioxin Furans	(*30*)
American alligator	↓ Testosterone (male) Abnormal testicular cells ↑ 17β-Estradiol (female) ↑ Polyovular follicles and polynuclear oocytes	Poor quality eggs	DDE	(*13*)
Birds				
Western gulls	Retained Müllerian ducts Abnormal gonadal morphology	Female–female pair bonds Abnormal mating behavior	DDT DDE	(*57*)
Bald eagle	↑ Embryonic mortality and deformities	↓ Fertility	PCBs DDE Dieldrin	(*58*)
Japanese quail	?	↓ 17β-Estradiol before sexual maturation Delayed oviposition ↓ Laying capacity	PCBs	(*59*)
Mammals				
Dall's porpoises	?	↓ Plasma testosterone	*p,p'*-DDE	(*60*)
Beluga	?	↓ Follicular activity Mammary carcinoma	DDT Mirex PCBs	(*61*)

[a]Partial, representative listing only. [b]As the mechanisms underlying many of these effects are unknown or under study, the phenomenon listed as activational may be due to an organizational effect not apparently obvious at birth. [c]The contaminant(s) listed has been found to compose the greatest body burden in the animals studied.

For example, phytoestrogens in drying grasses decrease reproductive output in wild populations of California quail (*63*) and deer mice (*64*). Similarly, clover disease in livestock, caused by animals feeding on phytoestrogen-containing subterranean clove (*Trifolium subterraneum*), induces infertility in adults (*65*). These examples, which represent extremes in the action of phytoestrogens, and studies demonstrating that some plant compounds are antiestrogens, are used by some to suggest that environmental estrogens represent no generalized health threat because organisms are exposed to estrogen mimics or antiestrogens everyday via natural foods. There is a flaw in this argument as it ignores the central paradigm of biology—evolution.

Plant–animal interactions have existed for eons, and evolutionary theory predicts that plants should battle herbivores because they represent predators (*66–68*). Compounds that would limit herbivore reproduction would decrease predation pressure on plants. Thus, clover disease is predicted as a natural ongoing evolutionary event. Why then do animals not respond to more plant secondary compounds in such a dramatic fashion? Evolutionary theory also predicts that those animals that are not affected by the plant phytoestrogens will reproduce at a greater rate and thus, given numerous generations, they will produce a population in which most individuals either avoid or adapt to any given chemical. Indeed, the phytoestrogen genistein only elicits estrogenic effects during specific windows of development (*69*) and, thus, chronological adaptation is a plausible mechanism of avoiding the estrogenic effects of phytoestrogens. Additionally, humans physiologically respond to the exposure of the phytoestrogen genistein by stimulating sex-hormone binding globulin production (*70*) and suppressing aromatase activity (*71*), both of which reduce the amount of bioavailable natural estrogen.

Physiological adaptation to an environmental toxic compound (acquired resistance) is seen in insect pests responding to pesticide exposure and bacteria responding to antibiotic treatments. We can record these examples of evolutionary response due to these organisms' very short generation times relative to our own generation length. An extension of the acquired resistance model suggests that vertebrates should show a similar pattern, and it is clear that we do (*72*). We eat many plant compounds that are toxic, and several act as endocrine disruptors, such as antithyroidal goitrogens found in many flowering vascular plants of the family Brassicae (e.g., cabbage, brussel sprouts, rutabaga, turnips) (*73*). But we readily degrade many of these substances metabolically so that the compound ingested and the subsequent breakdown products of this metabolism have minimal or no affect on our bodily processes. Moreover, we do not bioaccumulate these compounds. However, one cannot expect acquired resistance to evolve in vertebrates within a generation or two or maybe ever. The vertebrates living on earth today are exposed to a greater range of foreign chemicals than probably at any time in our evolutionary history. Although many of these synthetic compounds are metabolized and flushed from the body, many bioaccumulate. Thus, a situation exists where a developing egg or embryo is exposed to chemicals stored over a mother's lifetime. If these bioaccumulated compounds act as hormonal mimics, then embryonic development can be modified. These changes may be subtle, as discussed above, but can lead to catastrophic changes later in life.

Free versus Bound Hormone. The toxicokinetics of any given EDC are extremely complex, but several generalities can be made about the distribution and activity of such contaminants. As discussed earlier, lipid-soluble contaminants are mobilized from fat stores during energetically expensive reproductive events. These contaminants are mobilized to the extracellular fluid surrounding fat stores and are either localized into an adjacent organ or transported into the circulatory system. In the case of direct organ exposure, passage into the cell's cytoplasm and nucleus is unhindered as the lipophyllic compound passes easily through the cell and nuclear membranes. The kinetics are much more complicated for EDCs entering the circulatory system because the biological activity of a particular compound is not dependent on the amount of compound in the circulation, but rather the amount of compound available to cells.

When dealing with vertebrate hormones, most of a given hormone is usually bound to plasma proteins while a very small amount circulates in a free form. The plasma protein–hormone complexes are large and cannot cross capillary walls. Thus, only the unbound portion of hormone in the blood determines the hormone's physiological activity—an idea termed the free hormone hypothesis (*74*). In essence, plasma proteins in mammals act as storage depots for steroid hormones, but do these same plasma proteins protect tissues from EDCs? An answer can only come from analyses of specific contaminant–protein complexes, but most research on the binding of xenobiotic chemicals to plasma proteins has been conducted on drugs, not contaminants (*75*). This research has revealed basic information that can be used, such as the binding characteristics of albumin. Albumin has six binding regions and acts as a transport protein for numerous endogenous and exogenous compounds (*76*). However, albumin's equilibrium constant for steroid complexes is relatively low (*77*); thus, albumin offers little protection as a storage protein. Conversely, some plasma proteins avidly bind to contaminants as exemplified by the organochlorine pesticide dieldrin; 99% of dieldrin in circulation binds to plasma proteins (*78*). A new technique can assess the cellular availability of specific contaminants in the blood (*79*). This assay allows one to determine if plasma constituents bind specific EDCs and restrict their availability to a cell. Using this assay, it has been demonstrated that *o,p′*-DDT and its structural relative methoxychlor have very different accessabilities. *o,p′*-DDT in plasma is readily available to the cells whereas methoxychlor is not. This study emphasizes that plasma constituents (proteins or lipids) can modify the accessibility of an EDC to a cell and that even structurally related compounds may act and interact in plasma in significantly different ways. It is clear that future research should investigate the binding of plasma proteins or lipids to specific contaminants. If we hope to compare circulating levels of hormones and contaminants, it is critical that all factors involved in cellular exposure be considered.

Future Research Needs

The activational effects of steroid hormones on vertebrate reproductive, immune, and neurological systems are well established, but specific mechanisms by which steroids and endocrine-disrupting contaminants (EDCs) elicit organizational effects on these systems are lacking for most wildlife. This is especially apparent when one examines species other than mammals and birds. For instance, the mechanisms by which EDCs alter thyroid hormone concentrations in fish (*53,80*) and common seals (*81*) are as yet unknown. Both the magnitude and timing of EDC exposure should be considered when organizational effects are examined. Future studies must examine which

receptors bind EDCs and their subsequent endocrine action. For example, does a specific EDC bind to the estrogen or androgen receptor and is it an antagonist or agonist? In particular, this information is needed for those chemicals that are found commonly in wildlife exhibiting symptoms of EDC exposure. Information on receptor-related mechanisms will allow the development of assays for specific biomarkers and direct laboratory-based studies to link symptoms with contaminant exposure. Additionally, data must be obtained to determine if current models using steroid receptors obtained from mammalian species are characteristic of receptors from other species. Given the large degree of homology among steroid receptors from different species, it is likely that the mammalian model will provide a very useful tool, but this must be determined.

One aspect of the EDC problem that must be studied in more depth in nonmammalian vertebrates is the degradation, recycling, and plasma partitioning of various chemicals. For example, we know that reptiles bioaccumulate and biomagnify contaminants, and these contaminants are transferred to developing eggs. However, do reptiles, or any nonmammalian vertebrate, degrade various EDCs in a manner similar to traditional mammalian models? What is the difference in degradation or recycling of EDC between sexes? Oviparous females mobilize large amounts of lipid and protein during follicular development. The yolk protein, vitellogenin, is synthesized in the liver and circulates in the blood at very high concentrations. Does vitellogenin serve as a protein carrier of EDCs, thus transporting these contaminants into the eggs, as suggested that it does for natural steroids? Do other plasma proteins or lipids transport EDCs and protect them from hepatic degradation? What is the affinity of EDCs, if any, with sex hormone-binding globulin (SBG), corticosteroid-binding globulin, or plasma albumin? Although SBGs have been identified in reptiles, as in other nonmammalian vertebrates, their role is still poorly identified under even natural endocrine functioning. We must determine what role, if any, SBGs and other plasma constituents play in protecting and transporting EDCs. The biological activity of various EDCs will be dictated by the amount that is free or unbound and available to the developing embryo. Finally, additional research to thoroughly examine the physiological adaptations to various phytoestrogens, especially in an evolutionary context, would provide great insight into mechanisms by which vertebrates have adapted to naturally occurring endocrine-disrupting chemicals. Such adaptations may suggest methods whereby we can maximize the health of exposed wildlife populations.

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